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A comparative study of populations of *Ectopleura crocea* and *Ectopleura ralphi* (Hydrozoa, Tubulariidae) from the Southwestern Atlantic Ocean

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Abstract

Ectopleura crocea (L. Agassiz, 1862) and *Ectopleura ralphi* (Bale, 1884) are two of the nominal tubulariid species recorded for the Southwestern Atlantic Ocean (SWAO), presumably with wide but disjunct geographical ranges and similar morphologies. Our goal is to bring together data from morphology, histology, morphometry, cnidome, and molecules (COI and ITS1+5.8S) to assess the taxonomic identity of two populations of these nominal species in the SWAO. We have observed no significant difference or distributional patterns between the so-called Brazilian *E. ralphi* and Argentine *E. crocea* for both morphological and molecular data. Therefore, SWAO populations of *Ectopleura* belong to the same species. In a broader view, it is difficult to find decisive character distinguishing *E. crocea* from *E. ralphi*, and both species have indeed recently been synonymized, with the binomen *E. crocea* having nomenclatural priority. Geographically broader genetic analysis should be carried out in order to test the validity of this synonymy because taxonomical procedures such as studying type specimens and documenting broad phenotypic variability have not yet been conducted.

Key words: Tubulariidae, taxonomy, morphometry, cnidae, nematocysts, DNA analyses, COI, ITS1+5.8S

Introduction

Members of the family Tubulariidae inhabit shallow waters of all oceans, and they are commonly used in ecological, experimental, and morphological studies (Petersen 1990). Four phylogenetic analyses including representatives for the family have been conducted (Petersen 1990, Marques & Migotto 2001, Schuchert 2010, Nawrocki & Cartwright 2012). However, it still has a complex taxonomy (Migotto & Silveira 1987), mainly because of historical mistakes, incomplete information, and incorrect or incomplete understanding of intraspecific variation of its characters, such as polyp size, number of tentacles, morphology of gonophores, and ectodermal structure of the hydrocaulus (Tardent 1980, Petersen 1990).

Two nominal species of tubulariids recorded for the Southwestern Atlantic Ocean (SWAO), but presumably with wide geographical ranges (Figure 1), *Ectopleura crocea* (L. Agassiz, 1862) and *Ectopleura ralphi* (Bale, 1884), have quite similar morphologies (Ewer 1953, Brinckmann-Voss 1970, Millard 1975, Petersen 1990). They have a complex taxonomic history, and have previously been assigned to *Tubularia* (e.g., Genzano, 1998, 2001, 2005), *Ectopleura* (e.g., Migotto & da Silveira 1987), and *Pinauay* (Marques & Migotto 2001), until the most recent phylogenetic data has revealed that they would belong to the genus *Ectopleura* (Nawrocki & Cartwright 2012). In the SWAO, populations of the nominal species *E. ralphi* occur in shallow waters of southern Brazil (from the State of Espírito Santo, 20ºS, to Rio Grande do Sul, 29ºS—Migotto *et al.* 2002, and references therein), living on rocks, epizootically (on tunicates, sponges and bivalves), and on artificial substrates (Migotto & da Silveira 1987, Migotto *et al*. 2001, pers. obs.). On the other hand, the nominal species *E. crocea* occurs in shallow waters all

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over the Argentina coast (35–55ºS) (Genzano 1994, 2001, Genzano & Zamponi 1997, Oliveira *et al.* submitted), although populations have also been hypothesized as a cryptogenic species for that region (Orensanz *et al.* 2002).

Orensanz *et al.* (2002) called attention to the need for reviewing records of *E. crocea* from the SWAO and, recently, the Brazilian *E. ralphi* was referred as *E. crocea*, but this reassignment was not justified by the authors (Miranda *et al.* 2011). The morphological similarity between the species (Ewer 1953, Brinckmann-Voss 1970, Millard 1975, Petersen 1990), their parapatric distribution in the SWAO (cf. Genzano & Zamponi 1997, Migotto *et al.* 2002), and their putative cryptogenic status (Orensanz *et al.* 2002), clarify the need to review *E. crocea* and *E. ralphi* from the SWAO. Besides, when the two nominal species were recently synonymized (Bouillon *et al.* 2006, Schuchert 2010) under the binomen *E. crocea*, the hypothesis was not based on geographically broader genetic and phenotypic analyses.

Therefore, the goal of this study is to bring together data from morphology, histology, morphometry, cnidome, and molecules (COI and ITS1+5.8S) to assess the taxonomic identity of these two nominal species in the SWAO and discuss consequences of their synonymy.

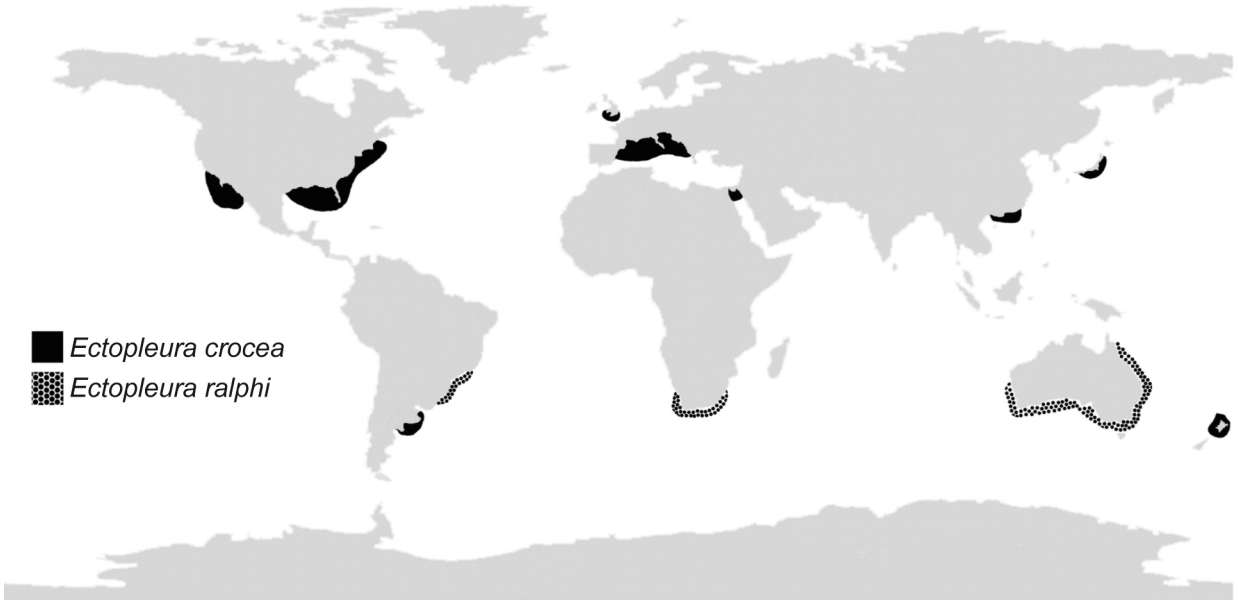

FIGURE 1. Worldwide distribution of *Ectopleura crocea* and *Ectopleura ralphi* (data from Agassiz 1862; Allman 1871; Allen 1900; Torrey 1902; Stechow 1907, 1925; Hargitt 1927; Ewer 1953; Millard 1959, 1966, 1975; Yamada 1959; Rees 1963; Brinckmann-Voss 1970; Calder 1971; Schmidt 1971; Watson 1980, 1982; Migotto & da Silveira 1987; Hirohito 1988; Petersen 1990; Schuchert 1996; Bouillon *et al*. 2004; Genzano 2005).

Material and methods

Material. The studied specimens from Brazil (States of Rio de Janeiro, São Paulo, Paraná, and Santa Catarina) and Argentina (Province of Buenos Aires) (Figure 2) are deposited in the collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP). Specimens fixed in formalin were used for morphology, cnidome, and morphometry. The DNA study was based on 22 specimens preserved in 95% ethanol (State of Rio de Janeiro, five samples; State of São Paulo, five samples; State of Paraná, five samples; State of Santa Catarina, three samples; Argentina, Mar del Plata, four samples).

Morphology. The characters used for the morphological assessment were those from previous descriptions (Agassiz 1862, Torrey 1902, Stechow 1925, Ewer 1953, Brinckmann-Voss 1970, Calder 1971, Schmidt 1971, Millard 1975, Watson 1980, Migotto & Silveira 1987, Hirohito 1988, Petersen 1990, Schuchert 1996, 2010, Bouillon *et al*. 2004). Animals were studied using light microscopy (simple and compound microscopes), scanning electronic microscopy (SEM), and histology. SEM images were obtained using a Carl Zeiss DSM 940, after standard protocols (Migotto & Marques 1999). Histological transversal sections of polyps on hydranths, hydrocaulus, oral and aboral tentacles were stained with hematoxylin of Weigert (HEW) and acid fuchsine (standard protocol in Marques 2001).

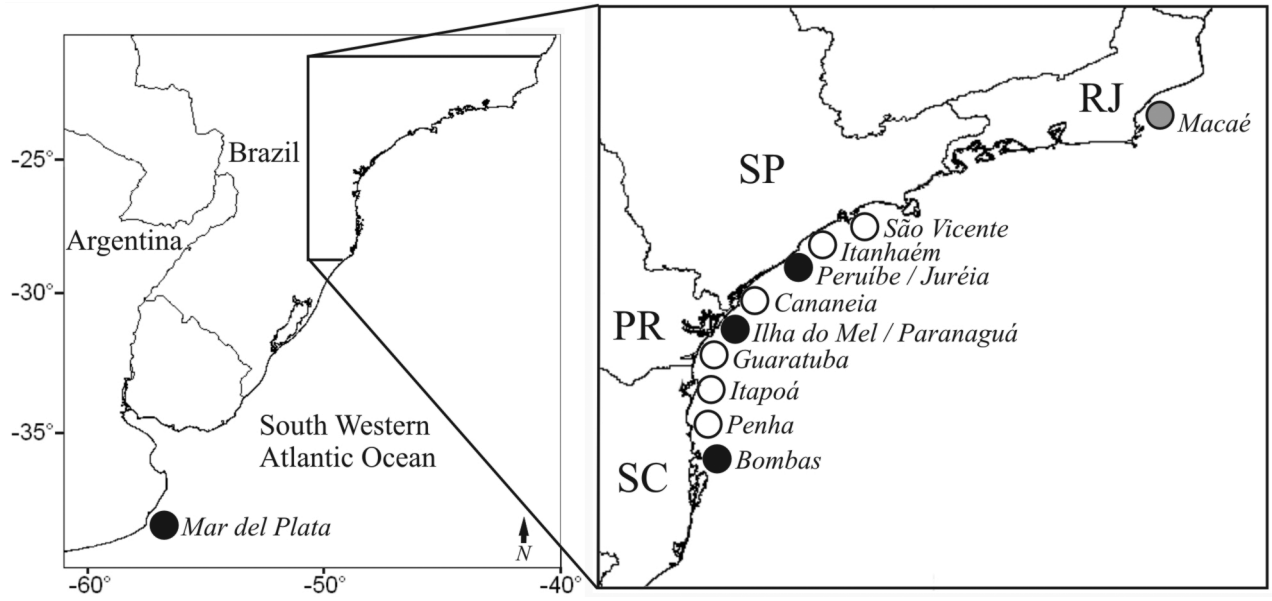

FIGURE 2. Sampled localities of the specimens used in morphological and molecular analyses. White circles are localities whose samples were used in morphological analyses; black circles for both morphological and molecular analyses; grey circle for molecular analyses only. RJ—State of Rio de Janeiro; SP—State of São Paulo, PR—State of Paraná, SC—State of Santa Catarina.

Morphometry was carried out considering the length/width of hydrocaulus, hydranths and gonophores, length of tentacles, and the cnidome. The identification of the nematocysts is based in previous classifications (Weill 1934, Mariscal 1974). Length/width of 20 undischarged capsules for each type of nematocyst from oral and aboral tentacles were measured for five different hydranths per locality. Morphometric assessment was based on principal components analyses (PCA) carried out in the software MVSP 3.12d (http://www.kovcomp.co.uk/mvsp).

DNA data and analyses. Total genomic DNA was extracted with Instagene (Bio-Rad) using one entire hydranth. DNA segments were amplified using PCR, with a total volume of 25 µl per reaction, and using the kits Taq Fermentas or pure Taq Ready-to-Go PCR beads (GE Healthcare). PCR primers were LCO12131 (gttttahtdggagggtttgg; this study) + HCO2198 (Folmer *et al*. 1994) for COI and ITS1f + ITS1r (Schroth *et al.* 2002) for ITS1+5.8S. The primer LCO12131 was designed based on a data set of 161 COI sequences representing 25 medusozoan species, and the code 12131 refers to the position of the 5' end of the primer on the COI region of the mitochondrial genome of *Aurelia aurita* (Scyphozoa, Semaeostomeae) (GenBank accession number DQ787873).

PCR products were purified with AMPure® magnetic column kit (Agencourt®). DNA sequencing was made using Big Dye® Terminator v3.1 (Applied Biosystems) and the same primers for PCR. DNA sequences were read in an ABI PRISM® 3100 genetic analyzer (Hitachi). Chromatograms obtained were analyzed by eye and no double peaks were detected. Megablast analyses (Zhang *et al*. 2000) were conducted in GenBank database to test for contaminants or nuclear pseudogenes. COI sequences were translated to amino acid (a.a.) using the Coelenterate mitochondrial code, and confirmed with the corresponding protein from *Palythoa tuberculosa* (Anthozoa, Hexacorallia) (GenBank accession number BAE48388). COI and ITS1+5.8S sequences were aligned in BioEdit (Hall 1999) using its implementation of ClustalW (Thompson *et al.* 1994). GenBank accession numbers for the obtained sequences are JX898187–JX898197 (COI) and JX898198–JX898203 (ITS1+5.8S).

Haplotype relationships were depicted from parsimony haplotype networks, constructed in TCS v.1.21 (Clement *et al.* 2000). Molecular syntheses include data on polymorphic sites, synonymous and non-synonymous substitutions and nucleotide diversity (π) obtained with DnaSP 5.10 (Librado & Rozas 2009). Significance of genetic differences between sampling points were estimated by analyses of population pairwise Fst and corrected average of pairwise differences, using Arlequin v.3.1 (Excoffier *et al*. 2005). For the purpose of this work, only unique haplotypes found for each locality were considered for the analyses.

Results

Taxonomic account

Family Tubulariidae Goldfuss, 1818

Subfamily Ectopleurinae Marques & Migotto, 2001

***Ectopleura crocea* (L. Agassiz, 1862)**
(Figures 3–5; Tables 1–8)

Parypha crocea L. Agassiz, 1862: 249, pls 23-2a.
Tubularia mesembryanthemum Allman 1871: 418, figs. 83–84; Hargitt 1927: 494; Yamada 1959: 16; Schmidt 1971: 32, pl. 2B; Hirohito 1988: 18, fig. 4, pl.1, fig. B.
Tubularia crocea; Torrey 1902: 42, pl. 3, figs. 22–23; Rees 1963: 1223; Brinckmann-Voss 1970: 28, text-fig. 30–34; Calder 1971: 24, pl. 1C; Genzano, Cuartas & Excoffon 1991: 69, pl. 5C; Blanco 1994: 182; Genzano & Zamponi 2003: 306, 307, 309, Tables 2-3; Demicheli & Scarabino 2006: 530.
Tubularia ralphi Bale 1884: 42; Watson 1980: 60, fig. 25–37; Watson 1982: 85, fig. 4.6.b, Plate 7.5.
Tubularia gracilis von Lendenfeld 1885: 597, fig. 51–52.
Tubularia sagamina Stechow 1907: 194; Yamada 1959: 16.
Tubularia australis Stechow 1925: 196.
Tubularia warreni Ewer 1953: 351, text-fig. 1–4; Millard 1959: 299; Millard 1966: 435; Millard 1975: 35, frontispiece, fig. 15A–G.
Ectopleura warreni; Migotto & Silveira 1987: 101, fig. 3; Migotto 1996: 25; Grohmann et al. 1997: 230, Table 1; Rosso & Marques 1997: 417, 420, 421, Table 1, Figure 4.
Ectopleura crocea; Petersen 1990: 174, fig. 27; Schuchert 1996: 107, figs. 64a–g; Schuchert 2001: 43; Bouillon *et al.* 2004: 104, fig. 55E–F; Genzano et al. 2009: 37, 40, Tables 2-3, Fig. 3; Schuchert 2010: 357-362, fig. 6.
Ectopleura ralphi; Petersen 1990: 175; Schuchert 1996: 109; Migotto, Marques & Flynn 2001: 289, 290, 293-297, figs. 5-6, Table 1.
Pinauay ralphi; Marques & Migotto 2001: 475, 478, 480, figs. 2B, 3, Table 1; Migotto, Marques, Morandini & Silveira 2002: 10; Marques & Migotto 1994: 173, 174, Tables 15.1, 15.2; Grohmann 2006: 103, 104, Tables 1-2; Oliveira, Marques & Migotto, 2006: 4, 10, fig. 5, Table 1; Oliveira & Marques 2007: 31; Grohmann 2007: page not numbered; Silveira & Morandini 2011: 5.
Pinauay crocea; Marques & Migotto 2001:480.

Examined material. **Brazil:** State of Rio de Janeiro, Macaé, Cavaleiros Beach, 22°24'S 41°47'W, 15.viii.2008, on rock, 95% ethanol, coll. A.C. Morandini (MZUSP 1633); State of São Paulo: São Vicente, Vacas Beach, 23º58S 46º23W, 05.ix.1991, intertidal fringe, on rock, under *Phragmatopoma* stripe, at the same level of *Eudendrium,* 4% formalin, coll. A.C. Marques (MZUSP 414); Itanhaém, Saudade Beach, 24º10'S 46º45'W, 26.viii.1991, intertidal fringe, on rock, under *Phragmatopoma* stripe*,* 4% formalin, coll. A.C. Marques (MZUSP 406); Peruíbe, Centro Beach, 24º19'S 46º58'W, 12.viii.1992, intertidal fringe, on rock, sheltered place, forming an abundant stripe below *Phragmatopoma*, 18ºC, 28‰, 4% formalin, coll. A.C. Marques (MZUSP 433); Peruíbe, Jureia-Itatins Ecological Station, 24°34'S 47°14'W, 15.ix.2008, on rock, 95% ethanol, coll. J.M.M. Nogueira (MZUSP 1636); Cananéia, Argolão Rocky Shore near São João Hill, 25º00'S 47º57'W, 25.viii.1992, intertidal fringe, on rock, 19ºC, 28‰, 4% formalin, coll. A.C. Marques (MZUSP 444); State of Paraná: Mel Island, Encantadas Rock, 25º34'S 48º18'W, 06.viii.1988, 4% formalin, coll. M.A. Haddad (MZUSP 1750); Paranaguá, Yacht Club Paranaguá, 25°30'S 48°29'W, 10.x.2007, on artificial substrate, 95% ethanol, coll. M.A. Haddad, (MZUSP 1637); Guaratuba, bottom trawling net, 4 km of shore, 25º52'S 48º33'W, 01.xii.2003, 4% formalin, coll. M.A. Haddad (MZUSP 1751); State of Santa Catarina: Itapoá, Itapema beach, 26º05'S 48º36'W, 04.vi.2004, 4% formalin, coll. M.A. Haddad (MZUSP 1752); Penha, on a culture of mussels, 26º45'S 48º38'W, 17.vi.2005, 4% formalin, coll. M.A. Haddad (MZUSP 1753); Bombas, Bombas Beach, 27º07'S 48º30W, 03.xii.2006, 4% formalin, coll. M.A. Imazu (MZUSP 1754) and 95% ethanol, coll. E. Ale, (MZUSP 1638). **Argentina**: Mar del Plata, Punta Cantera, 38º04'S 57º32'W, 26.i.2002, intertidal fringe, 4% formalin, coll. G. Genzano (MZUSP 1755), 95% ethanol, coll. G. Genzano (MZUSP 1639).

Type specimens: *Ectopleura ralphi*, type specimen lost, a neotype was proposed based on specimens from Australia, Victoria, Port Phillip, Yarra River Entrance Beacon, 03.iv.1977, 1–2m, on mussel and ascidia,

formaldehyde (NMV G3227) (Watson 1980). *Ectopleura crocea*, we found no reference concerning the material described by L. Agassiz, from the port of Boston. It may be lost.

Description. Colonies dioecious, up to 55 mm high. Hydrorhiza and hydrocaulus with well-developed perisarc. Unbranched erect hydrocauli arising from stolonial hydrorhiza. Hydrocaulus' coenosarc split into two longitudinal chambers with basal diameter 200–420 µm, apical 340–1000 µm; distal region of hydrocaulus with globular expansion supporting terminal hydranth. Hydranth with one whorl of aboral and one whorl of oral tentacles; oral tentacles adnate to hypostome up to the mouth region, circular in transversal section; aboral tentacles quadrangular in transversal section. Unbranched blastostyles of gonosomes arising immediately above aboral whorl of tentacles; main axis of each blastostyle supporting gonophores. Female gonophore cryptomedusoid, oval, with eight distal laterally compressed crests surrounding terminal aperture, terminal region of spadix projecting to outside. Male gonophore cryptomedusoid, spherical to oval, without distal crests. Early released actinulae with 8–11 aboral capitate tentacles.

Aboral tentacles with four types of nematocysts: O-basitrichous isorhizas, rare and not measured (Figure 3A); basitrichous isorhizas, common, 6.45–12.19 X 2.84–6.04 µm (Figure 3B); desmonemes, abundant, spherical to oval, 3.50–6.81 X 2.41–5.13 µm (Figure 3C); small stenoteles, abundant, 5.02–7.82 X 3.66–8.09 µm (Figure 3E). Oral tentacles with three types of nematocysts: O-basitrichous isorhiza, rare and not measured (Figure 3A); basitrichous isorhizas, rare, 7.06–14.73 X 2.87–7.1 µm; large stenoteles, abundant, 7.36–12.5 X 6.07–11.38 µm and small stenoteles 4.93–7.93 X 3. 2–7.13 µm (Figure 3D).

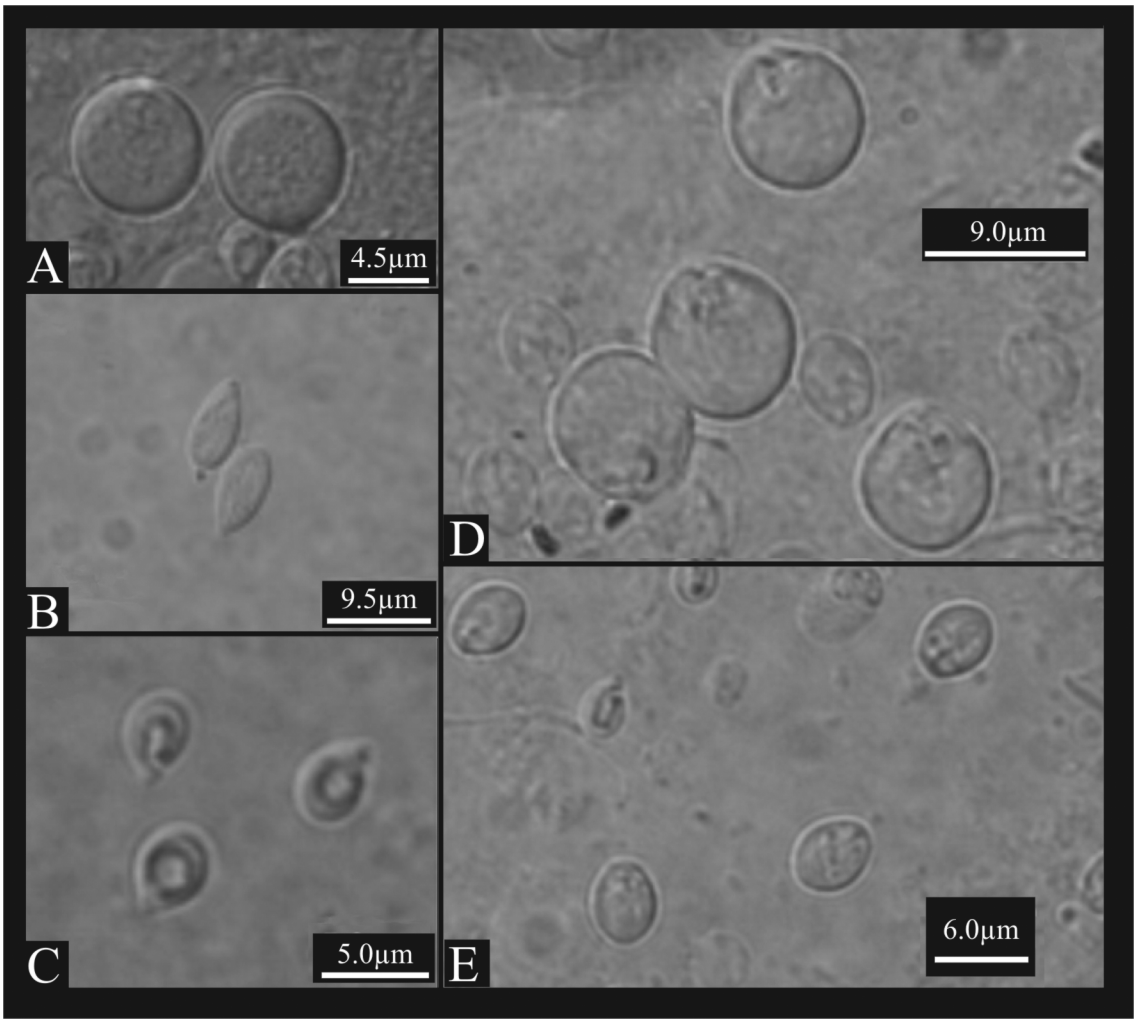

FIGURE 3. Different types of nematocysts documented under light microscopy. A: O-basitrichous isorhizas, B: basitrichous isorhizas, C: desmonemes, D: large stenoteles, E: small stenoteles.

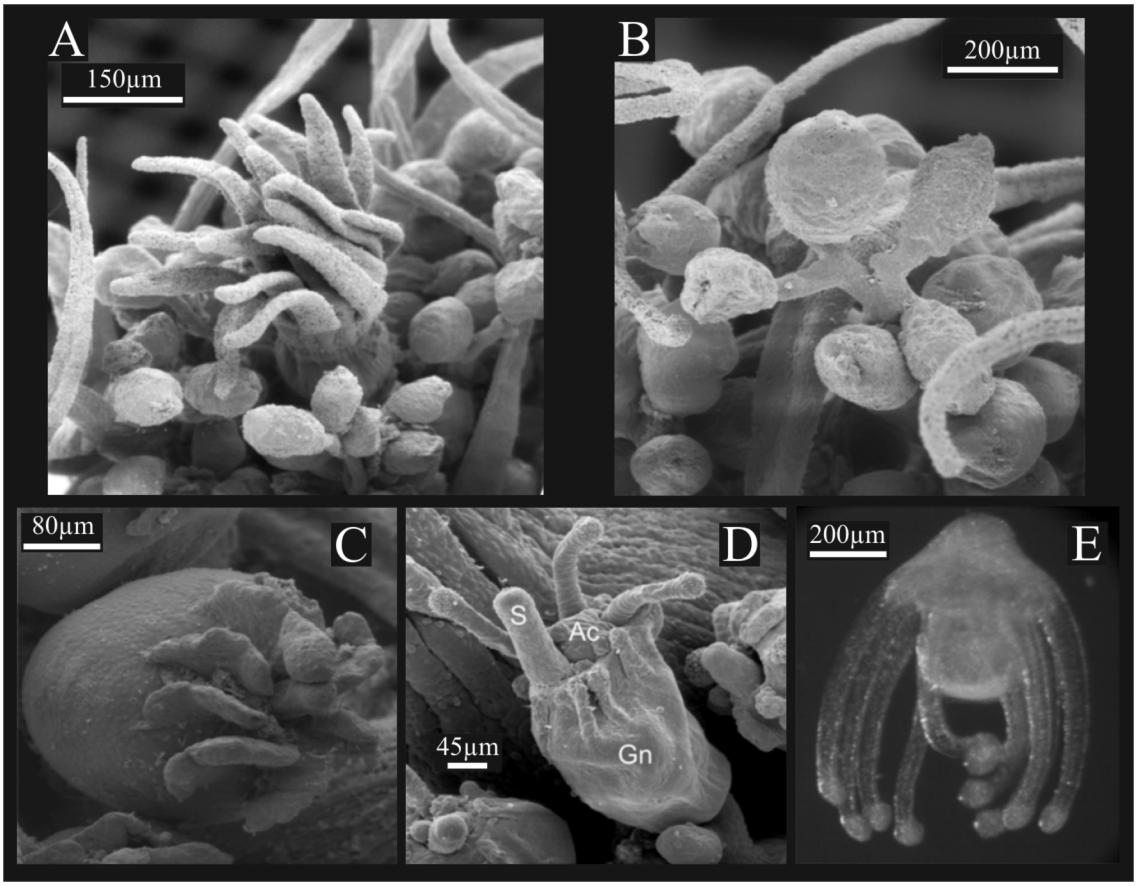

FIGURE 4. Morphological traits of gonophores (A–D, scanning electronic microscopy) and actinulae (E, light microscopy). A: Oral-lateral view of the hydranth with its blastostyles of male gonophores. B: Detail of the blastostyles with male gonophores. C: Female gonophore with the apical crests on top. D: Female gonophore liberating an actinula. E: Liberated actinula with capitate aboral tentacles. Legends on figures: Gn, gonophore; Ac, actinulae; S, spadix.

Distribution in the South Western Atlantic Ocean. Brazil: States of Espírito Santo (Grohmann *et al.* 1997, Grohmann 2006), São Paulo (Migotto & da Silveira 1987, Migotto 1996, Rosso & Marques 1997, Migotto *et al.* 2001, 2002, Marques & Migotto 2004, Oliveira *et al.* 2006, Oliveira & Marques 2007, Silveira & Morandini 2011), Paraná (Haddad, 1992), Santa Catarina (Miranda *et al.* 2011) and Rio Grande do Sul (Migotto & Silveira 1987) (see Migotto *et al.* 2002, Marques *et al.* 2003). Uruguay (Demicheli & Scarabino 2006). Argentina: Provinces of Buenos Aires (Blanco 1994, Genzano *et al*. 1991, 2009, Genzano 1994, 1998, Genzano & Rodriguez 1998), Río Negro and Chubut (Blanco 1994, Genzano et al. 1991), Santa Cruz and Tierra del Fuego (Oliveira et al. submitted).

Remarks. General morphology. The species *E. crocea* and *E. ralphi* are morphologically similar and have been considered sister-taxa (Marques & Migotto, 2001) or synonyms (Bouillon *et al.* 2006, Schuchert 2010). Historically, subtle differences have been cited to differentiate the species (Table 1). For instance, Petersen (1990) differentiated the two species by describing *E. crocea* as having more aboral and oral tentacles than *E. ralphi*. However, this relationship appears to vary (Table 1). Indeed, characters related to the tentacles are generally variable (Tables 1–2; see also Agassiz 1862, Hargitt 1927, Ewer 1953, Calder 1971, Schmidt 1971, Migotto & Silveira 1987, Hirohito 1988).

The SWAO specimens all have hydroucauli that broaden distally (Table 3), as described for *E. crocea* (Hirohito 1988, Schuchert 1996), although specimens with the same diameter throughout the hydrocaulus were reported by Petersen (1990). Each polyp is gonochoristic, although settlement of actinulae on already developed hydrocauli (Rungger 1969) may promote pseudo-hermaphroditism, a strategy also reported for other hydroids (Brinkmann-Voss 1970, Sommer 1990, Marques 2001, Schuchert 2010, Nawrocki & Cartwright 2012).

There are inconsistencies in the description of the blastostyles, either characterized as unbranched (Ewer 1953 and Millard 1966 for *E. ralphi*; Brinckmann-Voss 1970, Hirohito 1988, and Petersen 1990 for *E. crocea*) or

branched (Bale 1884, Millard 1975, Watson 1980, Migotto & Silveira 1987, and Petersen 1990 for *E. ralphi*; Schuchert 1996 for *E. crocea*) (Table 4). Descriptions of the neotype of *E. ralphi* from Melbourne (Australia) have different ways to describe the blastostyles, characterized either as "[...] only occasionally branched" (Schuchert 1996: 109; our underline) or as "mature blastostyles branched [...]" (Watson 1980: 61), or ("usually unbranched, but some branching can occur", Schuchert 2010, p. 359). Specimens from SWAO have unbranched blastostyles (Figure 4A–B), similar to those described by Allman (1871) and contrasting with the long bunches of gonophores described for other localities (Calder 1971, Millard 1975, Watson 1982, Petersen 1990). Millard (1959) commented on the difficulty of differentiating primary and secondary pedicels, which may explain the challenges of characterizing the ramification of blastostyles.

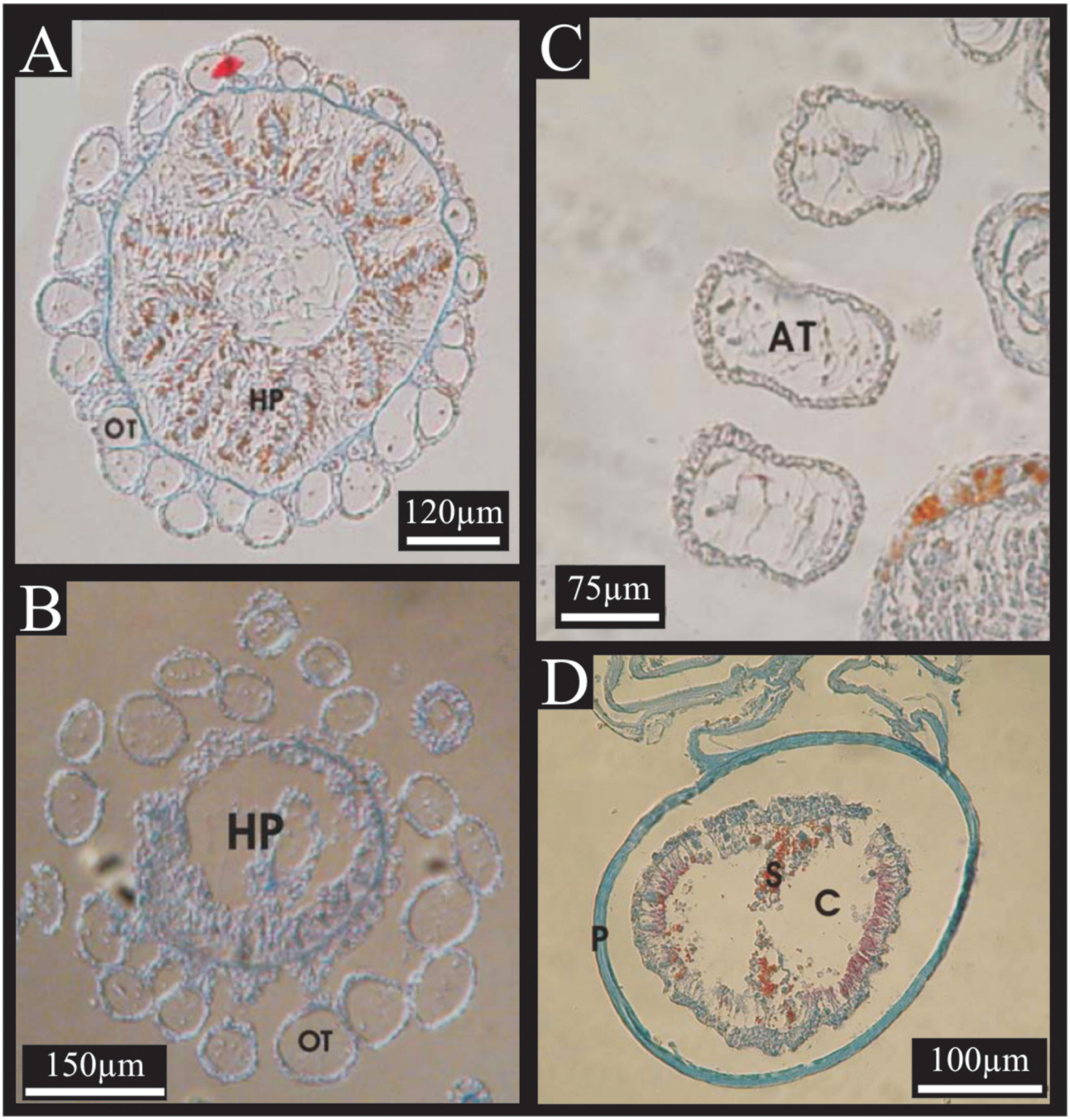

FIGURE 5. Histological sections documented under light microscopy. A: transversal section at the base of oral tentacles, where they are still adnate to hypostome. B: transversal section of the oral tentacles showing their circular shape. C: oblique section of the aboral tentacles showing the squared section of the cells constituting that. D: transversal section at distal region of hydrocaulus showing its division into two longitudinal chambers. Legends on figures: HO, hypostome; OT, oral tentacle; AT, aboral tentacle; S, septum; C, chamber; P, perisarc.

TABLE 1. Literature data of morphometric and meristic information for "*E. crocea*" (white rows) and "*E. ralphi*" (shaded rows), expressed as minimum–maximum values. Length and diameter are given in millimeters. T = *Tubularia*; E = *Ectopleura*.

Author (Original reference to species)	Hydrocaulus		Aboral tentacles		Oral tentacles		Locality
	length	diameter	number	length	number	length	
Agassiz (1862) (*Paripha crocea*)	63.5–88.9		24		24		Boston harbor, USA
Allman (1871) (*T. mesembryanthemum*)	101		20–24		24		Gulf of Spezia, Italy
Bale (1884) (*T. ralphi*)	76–101						Hobsons Bay, Australia
Allen (1900) (*Paripha crocea*)	63.5–76.2		16–24				Woods Holl, USA
Torrey (1902) (*T. crocea*)	inconstant		24 top		15–18		San Francisco and San Diego Bays, USA
Stechow (1907) (*T. sagamina*)	150		50	9.0	20–25		Misaki, Japan
Stechow (1925) (*T. australis*)	50–80		20		16–18		Fremantle, Australia
Hargitt (1927) (*T. mesembryanthemum*)	35–50		20–25		20–25		South China
Ewer (1953) (*T. warreni*)	100 top	0.22–0.8	24–30	4 top	22–27	0.8 top	Natal, South Africa
Millard (1959) (*T. warreni)*	50		18–29		15–24		Natal and East coast, Africa
Yamada (1959) (*T. mesembryanthemum*)	30		20		10		Sagami Bay and Seto, Japan; Amoy, South China
Yamada (1959) (*T. sagamina*)	150		50		20–25		Sagami Bay and Seto, Japan; Amoy, South China
Rees (1963) (*T. crocea*)	50						England
Millard (1966) (*T. warreni*)	17.5	1.0					East cost of South Africa
Brinckmann-Voss (1970) (*T. crocea*)			28 top		17–20		Italy, England and France
Calder (1971) (*T. crocea*)	100		20–24		20–24		Florida and north of Gulf of Mexico
Schmidt (1971) (*T. mesembryanthemum*)	50		20–25		20–25		Gulf Aqaba, Red Sea
Millard (1975) (*T. warreni)*	50–100		31 top	5 or more	27 top	1 or more	Durban Harbor, South Africa
Watson (1980) (*T. ralphi*)	120 top	0.3–0.5	16–27	4–5	15–25	2–3	Victoria, Australia
Watson (1982) (*T. ralphi*)	120		16–27		15–25		Fremantle, Australia
Migotto & da Silveira (1987) (*E. warreni*)	70 top	0.18–0.6	12–27	0.4–6.0	11–30	0.4–1.9	Southeastern and Southern coast, Brazil
Hirohito (1988) (*T. mesembryanthemum*)	40–50		16–31		14–43		Japan
Petersen (1990) (*E. crocea*)	70		22–30		18–24		East and West coast, USA
Petersen (1990) (*E. ralphi*)			16–27		15–25		Inkerman Creek, Australia
Schuchert (1996) (*E. crocea)*	50	0.2–0.6	22 top		20		New Zealand
Bouillon *et al.* (2004) (*E. crocea*)	70		22–30		18–24		Mediterranean
Schuchert (1996) (*E. crocea*)	30–80, maxi 120	0.6–0.8, but up 2	22–28 (max. 38)		ca. 18 (max. 26)		Australia, South Africa, USA (Atlantic), Mediterranean (Italy, France)

TABLE 2. Morphometric patterns of hydranth and tentacles for specimens of *Ectopleura crocea* per locality of occurrence in the southwestern Atlantic Ocean. Values are given as minimum–maximum measurements in millimeters, average±standard deviation in parentheses (*n=1).

	Hydranth		*Tentacles*			
Locality	length	diameter	aboral		oral	
(n)			number	length	number	length
São Vicente (10)	900–1600 (1288±204)	600–960 (745±126.43)	15–20	1300–2160 (1714±335.47)	15–19	500–800 (656±97.89)
Itanhaém (10)	1360–1800 (1552±184.08)	660–1200 (870±167.4)	17–20	1900–2700 (2180±264.83)	16–21	400–700 (612±102.07)
Peruíbe (10)	1440–2260 (1842±279.44)	800–1400 (1012±190.48)	16–20	2100–3200 (2494±326.33)	16–20	580–1100 (848±187.19)
Ilha do Mel (1)	1500	800	20	1700	18	500
Guaratuba (7)	1000–1200 (1133.33±115.47)	700–1000 (866,67±152.75)	21–23	1400*	15–23	500*
Itapoá (10)	1660–2700 (2048±328.93)	900–1300 (1064±138.5)	18–23	1700–3400 (2494±537.41)	15–20	800–1200 (957±105.63)
Penha (10)	1100–2000 (1620±311.98)	800–1200 (1000±141.42)	19–23	1800–2800 (2107.5±329.32)	16–18	500–800 (644.29±105.18)
Bombas (10)	980–1550 (1243±200.22)	460–800 (636±119.18)	14–18	1400–2000 (1688±172.87)	15–20	340–540 (452±56.73)
Mar del Plata (10)	1400–2900 (2208±492.09)	900–1500 (1199±178.29)	20–26	2520–4200 (3217±547.93)	17–19	700–1000 (825±116.07)

TABLE 3. Morphometric patterns of hydrocaulus and gonophores for specimens of *Ectopleura* per locality of occurrence in the southwestern Atlantic Ocean. Values are given as minimum–maximum measurements in millimeters, average±standard deviation in parentheses

	Hydrocaulus				*Gonophores*	
Locality	length	diameter			length	diameter
(n)		proximal	medial	distal		
São Vicente (10)	14–35 (20.7±6.17)	240–300 (280±23.09)	300–400 (350±28.67)	340–540 (436±65.18)	260–500 (368±68.77)	180–300 (228±39.1)
Itanhaém (10)	22–36 (29.1±4.65)	220–400 (304±52.32)	420–520 (452±30.11)	440–620 (528±68.77)	360–660 (482±88.67)	240–520 (362±85.09)
Peruíbe (10)	13–35 (21.3±6.02)	240–340 (284±30.98)	300–440 (372±53.5)	500–800 (604±95.59)	440–660 (524±79.33)	280–500 (388±76.71)
Ilha do Mel (1)	30	320	440	520	260	200
Guaratuba (7)	10–18 (14.17±3.13)	220–400 (306.67±70.05)	280–480 (383.33±68.61)	400–620 (513.33±97.71)	300–400 (545.71±41.17)	240–340 (277.14±33.52)
Itapoá (10)	25–38 (32±4.27)	200–340 (270±44.47)	320–400 (350±30.18)	600–1000 (716±121.76)	440–760 (598±119.05)	300–460 (368±48.26)
Penha (10)	20–30 (25.6±3.24)	260–300 (284±15.78)	340–460 (394±35.34)	500–720 (650±63.42)	300–520 (432±75.54)	240–380 (306±40.06)
Bombas (10)	17–44 (29.5±9.91)	240–420 (298±49.4)	280–560 (358±79.13)	340–460 (418±41.58)	320–500 (432±59.03)	240–380 (298±45.66)
Mar del Plata (10)	11–22 (16.7±3.53)	300–400 (340±31.27)	400–500 (442±35.84)	580–900 (710±107.6)	480–900 (632±144.28)	300–540 (424±78.77)

TABLE 4. Literature data of morphological and meristic information for reproductive structures and actinular traits for "*E. crocea*" (white rows) and "*E. ralphi*" (shaded rows), expressed as minimum–maximum values. T = *Tubularia*; E = *Ectopleura*. For information of the localities see table 1.

Author (Original reference to species)	Female gonophore	Male gonophore	Blastostyles	Actinulae
Agassiz (1862) (*Paripha crocea*)	6–10 crests	no crests	simples or branched	
Allman (1871) (*T. mesembryanthemum*)	8 apical processes	4 small tubercles		no oral tentacles
Bale (1884) (*T. ralphi*)		4 tubercles	branched	
Allen (1900) (*Paripha crocea*)	6–8 crests	no crests	branched	
Torrey (1902) (*T. crocea*)	8 crests top	spherical, smooth or with processes		
Stechow (1907) (*T. sagamina*)	8 tubercles			6 tentacless
Stechow (1925) (*T. australis*)		4 small tubercles		8 aboral, 5 oral tentacles
Hargitt (1927) (*T. mesembryanthemum*)				
Ewer (1953) (*T. warreni*)	8 crests	no crests	usually no branched	8 aboral tentacles
Millard (1959) (*T. warreni*)			primary and secondary pedicels hard to differentiate	6 oral tentacles
Yamada (1959) (*T. mesembryanthemum*)	5–6 irregular processes			
Yamada (1959) (*T. sagamina*)				
Rees (1963) (*T. crocea*)	6–8 processes			
Millard (1966) (*T. warreni*)			no branched	
Brinckmann-Voss (1970) (*T. crocea*)	6–8 processes	no crests		only aboral tentacles 4–7
Calder (1971) (*T. crocea*)	6–10 processes	oval or spherical, no crests	longs	
Schmidt (1971) (*T. mesembryanthemum*)	8 processes	4 tubercles		
Millard (1975) (*T. warreni*)	8 crests	smooth or with 4–5 rudiments	few lateral branches 6 oral tentacles top	5–12 aboral tentacles
Watson (1980) (*T. ralphi*)	8 crests	no processes	branched	oral tentacles rudiments 5–8; 8 aboral tentacles
Watson (1982) (*T. ralphi*)	8 crests	no crests	big bunches	8 tentacles
Migotto & da Silveira (1987) (*E. warreni*)	4–8 crests	no crests	branched	6–12 capitate aboral tentacles, oral rudiments
Hirohito (1988) (*T. mesembryanthemum*)	6–8 processes	smooth or with 4 small processes	no branched	
Petersen (1990) (*E. crocea*)	8 crests	smooth or rare apical processes	usually no branched	no oral tentacles
Petersen (1990) (*E. ralphi*)			branched and longs	5–8 oral tentacles
Schuchert (1996) (*E. crocea*)	6–8 crests	no apical crests	branched	8 aboral capitate tentacles
Bouillon *et al.* (2004) (*E. crocea*)	8 crests	4 rudimentary tentacles		no oral tentacles
Schuchert (1996) (*E. crocea*)	6-8 (rarely 10) processes	without crests	usually unbranched, but some branching can occur	4 oral tentacles in 1 out of 8 actinula, no oral tentacles in 7 other larva, and 8–10 aboral tentacles

The morphology of the female gonophores (Figure 4C) is a diagnostic feature for *E. crocea* (Rees & Thursfield 1965, Schmidt 1971, Petersen 1990, Schuchert 2001, 2010). Nevertheless, some variation may be observed, mainly because of the development of the gonophores, or due to their contraction (Torrey 1902, Schuchert 1996). It is hard to identify tubulariids without gonophores (Watson 1982), and this may be the cause of misidentifications between *E. crocea* and *Ectopleura larynx* (Ellis & Solander, 1786), especially for the Northwestern Atlantic (e.g. Fraser 1944).

Male gonophores do not present apical crests (Figure 4B; see also Agassiz 1862, Brinckmann-Voss 1970, Calder 1971, and Schuchert 1996, 2010 for *E. crocea*; Ewer 1953, Watson 1980, 1982, Migotto & Silveira 1987 for *E. ralphi*), although in some cases they are described with small apical processes, varying in size and development between colonies, and even in the same colony (Table 4) (Allman 1871, Hirohito 1988, and Petersen 1990 for *E. crocea*; Stechow 1925 and Millard 1975 for *E. ralphi*).

Larval characters, such as tentacles, were already used to separate *E. ralphi* and *E. crocea*. A vague note in Schuchert (1996: 109, appears to refer to the observations of someone else) states that "the actinulae of *E. ralphi*, however, are reported to have rudiments of oral tentacles which are absent from *E. crocea*". Other data refer to variations in morphology and number of tentacles of the actinulae (Ewer 1953, Brinckmann-Voss 1970, Millard 1975, Watson 1980, 1982, Migotto & Silveira 1987, Petersen 1990, Schuchert 1996, 2010; see Table 4). Presumably "the oral tentacles will develop anyway immediately after the release of the actinula and the presence or absence in liberated ones is thus only a matter of timing" (Shuchert 2010, p. 361), an interpretation that attenuates the importance of the variation. The actinulae of the SWAO present 8–10 (Brazil) or 8–11 (Argentina) capitate aboral tentacles, depending on their development, and do not have rudiments of oral tentacles (Figure 4D–E), but the variability of this character was never strictly assessed.

Detailed studies on anatomical and histological characters have corroborated previous observations. Among these, the histological preparations confirmed that the oral tentacles are circular in a transversal section (Figure 5A–B), while the aboral tentacles are squared (Figure 5C), as described by Petersen (1990) for *E. crocea*. Also, transversal sections of the hydrocaulus have shown that its coenosarc is split into two longitudinal chambers (Figure 5D), as already noted before (Ewer 1953 for *E. ralphi*; Allman 1871 and Schuchert 1996 for *E. crocea*), although this feature was considered to be inconstant in number and size (Millard 1959, 1975 for *E. ralphi*; Campbell & Campbell 1968, Hirohito 1988, and Petersen 1990 for *E. crocea*).

Cnidome. The cnidome was uniform throughout all studied populations from SWAO (Figures 3A, B, D, E). Literature data for *E. crocea* present few discrepancies (Table 5, contrasting with Table 6), for instance a cnidome restricted to stenoteles and desmonemes (Brinckmann-Voss 1970) and a potential contamination by microbasic euryteles (Schuchert 1996; in Schuchert 2010, p. 360, they are referred to "rare euryteles").

TABLE 5. Summary of literature data of cnidomes described for "*E. crocea"* (white rows) and "*E. ralphi"* (shaded rows). The nematocysts of *E. crocea* by Schuchert 2010 (p. 360) have the same dimensions of those by Schuchert (1996) and Millard (1975). Measurements in micrometers, min–max. (*): only length reported.

Author (Original reference to species)		Heterotrichous anisorhiza	Basitrichous isorhiza	Stenotele (large)	Stenotele (small)	Desmoneme	Microbasic eurytele
Ewer (1959)	length	9.5 *	9.0	8.0		5.0	
(*Tubularia warreni*)	width		8.0	7.0 (stenotele)		4.0	
Brinckmann-Voss (1970)	length			present		present	
(*Tubularia crocea*)	width						
Millard (1975)	length	7.8–9.6	7.8–9.0	4.8–10.8		4.2–5.4	
(*Tubularia warreni*)	width	7.2–9.6	2.4–3.0	4.2–9.6 (stenotele)		2.4–4.2	
Watson (1980)	length	7.0	7.0–9.0	6.0	5.6–6.0	4.0–6.0	
(*Tubularia ralphi*)	width	9.0	4.0–4.0	8.0	4.0–5.0	3.0–4.0	
Migotto & da Silveira (1987)	length	6.6–11.2	7.9–11.2	9.2–11.2	4.6–9.2	3.3–5.3	
(*Ectopleura warreni*)	width	5.3–10.6	3.3–5.3	7.3–9.9	3.3–8.6	2.6–4.6	
Schuchert (1996)	length	8.0–9.5	9.0–9.5	6.5–7.0	5.0–6.0	5.0–5.5	9.0
(*Ectopleura crocea*)	width	7.0–9.5	3.0–4.0	5.0–5.5	3.5–4.5	3.0–3.5	4.0

TABLE 6. Measurements of nematocysts, in micrometers, by locality. Minimum value–maximum value (average value±Standard deviation). Number of measurements for each cell of the table is 100, except for those marked with *. (*1 n=26, *2 n=2, *3 n=40, *4 n=20.)

			São Vicente	Itanhaém	Peruíbe	Cananéia	Ilha do Mel
	Basitrichous	length	8.58–10.74 (9.43±0.48)	6.45–10.41 (8.39±0.67)	7.77–12.12 (9.39±0.67)	8.45–10.55 *1 (9.41±0.46)	8.14–11.86 (9.76±0.8)
		width	3.11–5.03 (4.03±0.32)	2.84–4.9 (3.77±0.43)	3.11–4.89 (3.94±0.3)	3.38–5.65 *1 (4.34±0.53)	3.16–6.04 (4.32±0.57)
Aboral	Desmonemes	length	4.18–5.64 (4.85±0.3)	3.5–5.5 (4.59±0.43)	4.46–5.79 (5.1±0.33)	3.9–5.98 (4.85±0.38)	4.46–6.0 (5.19±0.29)
tentacles		width	2.62–3.84 (3.35±0.26)	2.41–4.11 (3.17±0.33)	2.84–4.48 (3.63±0.28)	2.69–4.62 (3.48±0.33)	2.94–4.54 (3.62±0.28)
	Stenoteles	length	5.57–7.34 (6.29±0.32)	5.59–7.22 (6.29±0.31)	5.31–7.43 (6.36±0.37)	5.45–7.82 (6.52±0.44)	5.48–7.64 (6.45±0.41)
	(small)	width	4.2–5.96 (4.93±0.31)	4.27–5.5 (4.85±0.27)	4.14–6.67 (5.07±0.4)	4.33–6.05 (5.06±0.36)	4.49–6.04 (5.27±0.32)
	Basitrichous	length	7.06–11.6 (9.32±0.74)	7.67–10.21 (8.67±0.4)	7.22–10.78 (9.46±0.63)	8.2–9.03 *2 (8.62±0.59)	7.68–11.97 (9.79±0.79)
		width	2.87–5.24 (3.95±0.46)	3.19–4.81 (3.91±0.31)	3.04–4.94 (3.91±0.37)	3.75–4.09 *2 (3.92±0.24)	2.94–5.67 (4.46±0.49)
Oral	Stenoteles	length	7.37–11.88 (9.72±0.89)	7.43–11 (9.53±0.58)	7.36–11.37 (9.68±0.68)	8.27–11.47 *3 (10.2±0.77)	7.99–11.72 (10.61±0.59)
tentacles	(large)	width	6.07–10.29 (8.36±0.86)	6.45–9.86 (8.23±0.66)	6.53–10.4 (8.81±0.71)	6.21–10.31 *3 (8.79±0.86)	7.07–10.42 (9.37±0.6)
	Stenoteles	length	5.22–7.31 (6.19±0.4)	5.34–6.96 (6.06±0.3)	5.52–7.93 (6.29±0.49)	5.66–7.2 *4 (6.4±0.37)	5.64–7.47 (6.42±0.38)
	(small)	width	4.24–6.5 (5.03±0.37)	4.1–6.45 (4.84±0.32)	4.33–7.02 (5.14±0.53)	4.34–5.82 *4 (5.06±0.34)	4.41–6.46 (5.3±0.45)

			Guaratuba	Itapoá	Penha	Bombas	Mar del Plata
	Basitrichous	length	8.04–12.19 (9.94±0.75)	8.91–11.91 (10.6±0.6)	8.65–11.56 (10.12±0.58)	7.37–10.61 (9.24±0.56)	7.81–10.43 (9.37±0.52)
		width	3.06–5.87 (4.41±0.5)	3.73–5.82 (4.69±0.4)	3.37–5.29 (4.4±0.4)	3.54–5.33 (4.28±0.37)	3.17–5.26 (4.35±0.43)
Aboral	Desmoneme	length	3.94–6.81 (5.14±0.53)	4.57–6.66 (5.46±0.39)	4.51–6.6 (5.67±0.41)	4.67–6.54 (5.68±0.44)	4.02–6.17 (5.2±0.37)
tentacles		width	2.53–4.49 (3.67±0.46)	3.2–5.13 (4.14±0.33)	3.2–4.9 (4.12±0.38)	3.2–4.85 (4.08±0.31)	3.08–4.36 (3.67±0.28)
	Stenotele	length	5.02–7.3 (6.19±0.47)	5.77–7.31 (6.62±0.3)	5.37–7.66 (6.47±0.42)	5.13–7.69 (6.4±0.42)	5.26–6.96 (6.17±0.32)
	(small)	width	3.66–5.69 (4.72±0.42)	4.83–8.09 (5.49±0.47)	4.3–6.06 (5.01±0.35)	4.27–6.04 (5.0±0.37)	4.24–5.78 (4.88±0.3)
	Basitrichous	length	7.9–11.16 (9.38±0.7)	7.72–13.05 (10.38±0.7)	7.94–11.38 (9.7±0.77)	7.8–11.34 (9.39±0.65)	7.65–14.73 (9.32±0.94)
		width	2.87–5.18 (4.21±0.45)	3.51–5.56 (4.55±0.47)	3.42–5.52 (4.28±0.41)	3.41–5.73 (4.23±0.4)	3.28–7.1 (4.24±0.55)
Oral	Stenotele	length	8.64–11.87 (10.31±0.58)	8.19–12.15 (10.29±0.84)	9.25–12.5 (10.8±0.56)	8.98–12.22 (10.3±0.63)	8.68–12.09 (10.42±0.71)
tentacles	(large)	width	7.64–9.87 (8.67±0.56)	7.05–11.16 (9.19±0.8)	8.19–11.38 (9.76±0.61)	7.64–10.38 (9.09±0.52)	7.69–10.49 (8.98±0.59)
	Stenotele	length	5.03–7.08 (6.12±0.41)	5.09–7.37 (6.4±0.46)	5.28–7.22 (6.32±0.38)	4.93–7.48 (6.18±0.47)	5.23–7.26 (6.19±0.37)
	(small)	width	3.2–3.2–6.00 (4.6±0.43)	4.21–7.13 (5.34±0.4)	4.43–5.78 (5.07±0.32)	3.9–6.02 (4.95±0.43)	4.05–5.84 (4.96±0.38)

The basitrichous isorhizas found in SWAO specimens were characterized for "*Tubularia larynx*" Ellis & Solander, 1786 as "pseudo-microbasic b-mastigophore" (Östman *et al*. 1995: 166), possibly because of the basal spines of the tubule, giving the false appearance of a shaft under light microscopy (Östman 1987). The types isorhiza/anisorhiza and basitrichous/b-mastigophore are sometimes suggested as overlapped categories (Cutress 1955, England 1991).

The cnidome has been suggested to be a valuable taxonomic tool, even in more restricted geographic scales (Östman *et al.* 1987), but for the SWAO region, neither cnidome, nor dimensions of the hydroid, present any kind of geographic structure. Specimens from the states of São Paulo (São Vicente, Itanhaém, Peruíbe e Cananéia) and Santa Catarina (Itapoá and Penha) have the smallest and largest nematocysts, respectively (Figure 6A; Table 6). Comparing general dimensions of the hydroid (Figure 6B; Tables 2-3), specimens from Guaratuba and Mar del Plata have the lowest and highest values, respectively.

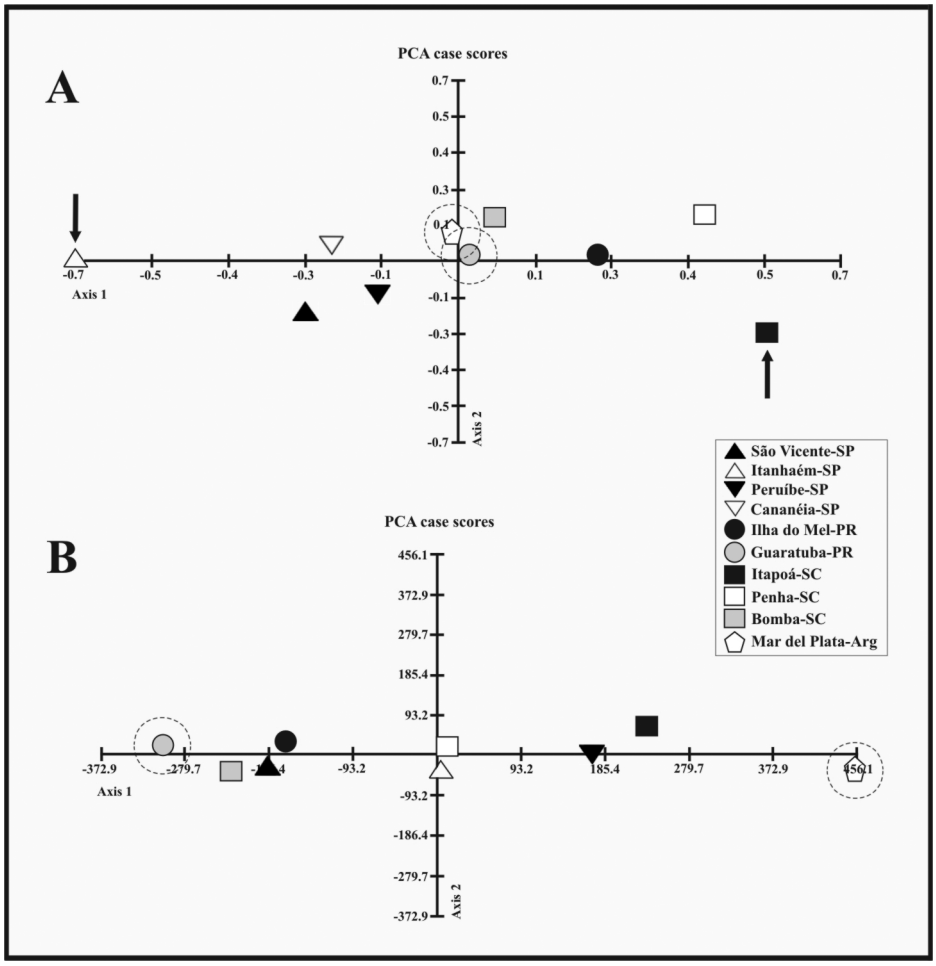

FIGURE 6. Principal component analyses (PCA) for cnidome (A) and morphology (B). Note that the minimum and maximum cases in A (arrows) do not match with minimum and maximum cases in B. Most similar cases marked by dashed circles.

DNA. Twenty-two specimens were analyzed for each marker. ITS1+5.8S (332bp) resulted in a total of six haplotypes, with the Argentine haplotype identical to that of Santa Catarina, both basically with a distance of three base pair indel from all others (Figure 7A). The genetic diversity found among the six haplotypes was only 0.1% (Table 7).

COI (489bp) resulted in a total of 11 haplotypes. Polymorphic sites revealed no saturation (Table 7), and 20 substitutions, with one synonymous transversion and four non-synonymous transitions (two replacements of Valine per Isoleucine, both aliphatic/hydrophobic; two replacements of aromatic Phenilalanine per aliphatic Leucine, both hydrophobic). These five changes were detected in only five Brazilian haplotypes. The COI haplotype network also shows low genetic distances between haplotypes (Figure 7B). It is interesting to notice that the genetic pairwise difference between the most distant sites (northernmost Macaé and southernmost Mar del Plata) is lower than those among closer intermediate localities (Table 8). Corrected average of pairwise differences and pairwise

fixation indexes between localities showed no significant values (Table 8). A recent publication included samples of *Ectopleura* from both South Africa (southern hemisphere, expected to be geographically more related to *E. ralphi*) and California (northern hemisphere, expected to be geographically more related to *E. crocea*), and found no genetic difference between these two populations (Nawrocki & Cartwright 2012).

TABLE 7. Summary of the genetic polymorphism observed for COI and ITS1+5.8S from the 22 samples of specimens of *Ectopleura* from the southwestern Atlantic Ocean in a total of five sampled localities.

	Length in bp	Haplotypes detected	Variable Sites	Mut[1]	Singl[2]	PIS[3]	Indel[4]	S/NS[5]	π/sd[6]
COI	489	11	20	20	8	12	0	15/5	0.012/0.001
ITS1+5.8S	332	3	1	1	1	0	3	-	0.001/0.0007

[1]Total number of mutations detected
[2]Singleton variable sites
[3]Parsimony informative sites
[4]Number of sites with insertion/deletion of bases
[5]Relation between synonymous/non-synonymous amino acid changes
[6]Nucleotide diversity/standard deviation

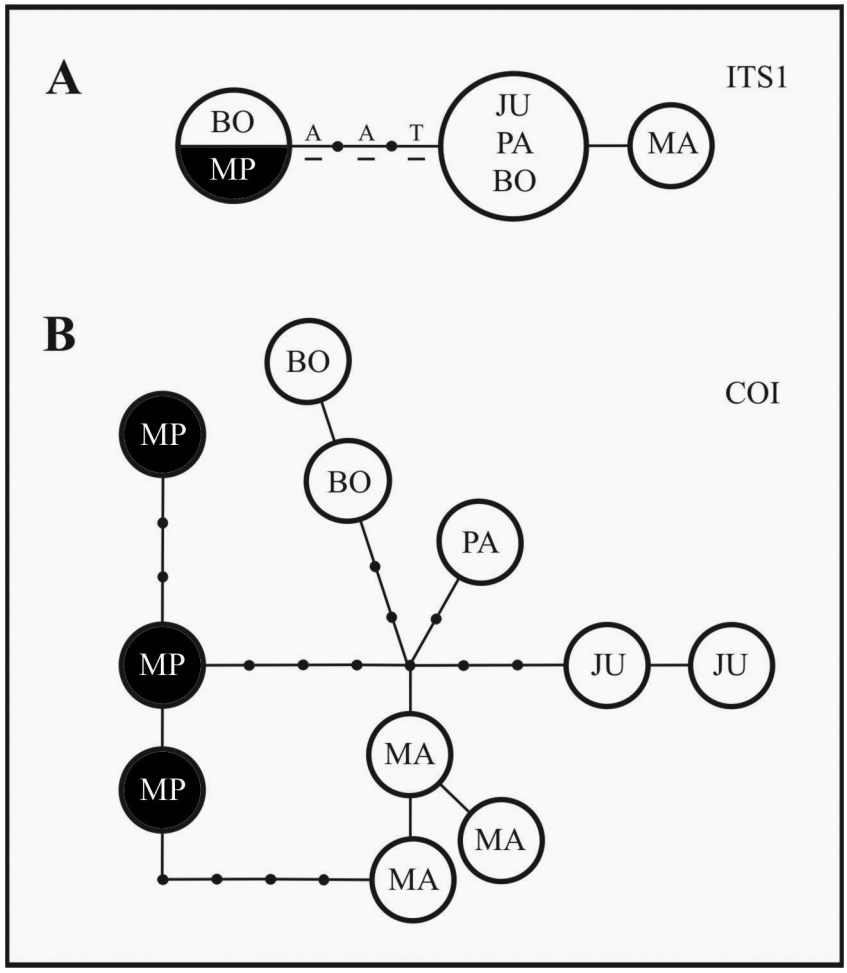

FIGURE 7. Haplotype parsimony networks for ITS1+5.8S (A) and COI (B) for the different populations of *Ectopleura crocea* and *Ectopleura ralphi* of the Southwestern Atlantic Ocean. Black circles for the Argentine haplotypes, white ones for Brazilian localities; the sizes of the circles are proportional to the number of localities sharing the haplotype; small black circles represent hypothetical or unsampled haplotypes. Lines connecting circles represent one substitution step. Note that A BO/MP samples differ from JU/PA/BO only by an indel of three base pairs (AAT/– – –). Locality codes are represented inside the circles as MA: Macaé, JU: Juréia, PA: Paraná, BO: Bombas, MP: Mar del Plata.

TABLE 8. Pairwise comparisons of localities for the CO1 marker (10,000 permutations) of specimens of *Ectopleura* from the southwestern Atlantic Ocean showing no significant genetic differences between them (p>0,05). Above the diagonal are the genetic distances expressed as corrected average of pairwise differences; below are the pairwise fixation indexes. Probability values are given in parentheses.

	Macaé (RJ)	Juréia (SP)	Paranaguá (PR)	Bombas (SC)	Mar del Plata (ARG)
Macaé (RJ)		4.00 (0.10)	3.00 (1.00)	4.00 (0.10)	4.78 (0.10)
Juréia (SP)	0.76 (0.10)		5.00 (1.00)	6.00 (0.33)	7.00 (0.10)
Paranaguá (PR)	0.64 (0.99)	0.82 (0.99)		5.00 (0.33)	6.00 (0.25)
Bombas (SC)	0.76 (0.10)	0.86 (0.33)	0.82 (0.33)		7.00 (0.10)
Mar del Plata (ARG)	0.70 (0.10)	0.76 (0.10)	0.64 (0.10)	0.76 (0.10)	

General discussion and concluding remarks. We have observed no significant difference or geographical patterns between Brazilian *E. ralphi* and Argentine *E. crocea* for both morphological and molecular data. Therefore, SWAO populations of *Ectopleura* likely belong to the same species.

Another important question is whether both species are valid. Schuchert (1996: 109) remarked that "until further, preferably also molecular, analysis has been made it seems advisable to keep both species separate". For Petersen (1990:176), "despite the obvious similarities between *E. ralphi* and *E. crocea* it seems best at present to keep the two species separate since the differences listed above appear to be constant and are shared by population of *E. ralphi* in both Australia and South Africa". Traditionally, the majority of the records of *E. crocea* and *E. ralphi* are from the Northern and Southern hemisphere, respectively (Figure 1). The fragmented and disjunct distributions of the species could also be explained by bioinvasion processes, either human or naturally mediated (Hewitt 2002, Ruiz *et al.* 2006, Marques, 2011, Mead *et al.* 2011, Rocha *et al.* 2013).

Considering our current knowledge of *E. crocea* and *E. ralphi*, we see no evidence to keep them apart. Considering them as synonymous, the binomen *E. crocea* would have nomenclatural priority. This was first proposed by Bouillon *et al.* (2006: 252) and reiterated by Schuchert (2010, that considered as a "new syn.", p. 357). The proposal by Bouillon *et al*. (2006) offered no concrete evidence or arguments supporting the synonymy. In contrast, Schuchert (2010) proposed a long synonymic list for *E. crocea*, but his analysis was actually based on very few specimens (n=12) from four restricted regions (Australia, South Africa, Mediterranean and Atlantic USA) – even in this restricted sample the phenotypic variability reported is impressive, which is also consistent with a composite of species (see Tables 1 and 4 for a summary). Schuchert (2010, p. 359) listed as diagnostic characters of *E. crocea* the "female sporosacs usually bearing six to eight crest-like processes around distal opening, several eggs or embryos per sporosac".

In this study we provide substantial evidence that two different SWAO "populations", previously assigned to *E. crocea* and *E. ralphi*, are the same. Nevertheless, whether these two different "populations" correctly represent the world nominal species *E. crocea* and *E. ralphi* is difficult to assess, and has to be considered conjectural. The proposed synonymy between *E. crocea* and *E. ralphi* (Bouillon *et al.* 2006, Schuchert 2010) have not been based on strict taxonomical procedures, i.e., neither were based on the study of the type specimen of *E. ralphi*, nor on broad geographic analysis considering extensive phenotypic variation of abundant material of the species, nor on broad molecular analysis from a wide geographical range. Therefore, the *ad hoc* proposal of the synonymy, even though presently accepted by us, has to be considered tentative and subject to further assessment.

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